

Implications for Human Health

by Leon Golberg*

To analyze the implications for human health, the toxicologist requires four sets of data: the results of toxicity and other studies in animals; quantitative data on actual or potential human exposure; whatever information is available on effects of exposure in man; and the statistical extrapolations from the dose-response relationships in animals to the (usually) much lower levels of human exposure. Professional expertise in toxicology is essential to assess the nature and severity of the toxic effects observed in animals, including such characteristics as potential for progression, irreversibility and production of incapacity. Given sufficient data, an estimate can be arrived at of the likelihood that such effects will be elicited in human populations of differing susceptibilities. The criteria by which the overall implications for human health can be judged comprise both the direct effects on man, as well as the indirect consequences stemming from environmental impacts.

In addressing an audience attuned to the emphases in today's media campaigns, a certain amount of 'deprogramming' is necessary. All toxicity is not cancer, nor birth defects. To the question: what else is there? — the answer is: a virtually infinite variety of other changes. On the one hand, a large number of organs and functions exist in the body that may be adversely affected by toxic agents. On the other, the environment of which man forms a part is not, and never was, a pristine background of "organic" existence generating zero incidence of adverse effects. That myth, so beloved by the romanticists, is not a reflection of reality. Furthermore, blind faith in statistical significance, following its conclusions wherever they may lead, is apt to spawn fallacies, as this audience well knows. One particularly heinous fallacy is the inference of causality from statistical association. Another is disregard of biological significance, that is, biologically-informed insight into the nature and causation of toxic effects. "Eyeballing" the data, and using perspective based on knowledge and experience — especially of the historical background of spontaneous pathological changes in laboratory animals — can contribute materially to interpretation of results and to sophisticated assessment of risk.

Throughout the course of toxicological investigations, the objective is to develop dose-response relationships for observed toxic effects and to en-

deavor to define the highest dose that does not elicit manifestations of toxicity in susceptible hosts. On the basis of this information, an appraisal of the implications for human health, that is, a risk assessment, is attempted. The process by which this conclusion is reached is summed up in Claude Bernard's equivalent of the classical "I came, I saw, I conquered": an observation is made; a comparison is established; and a judgment is rendered. For the purpose of this presentation, we start with the assumption that an observation has been made in laboratory animals, and consider the basis upon which the scientist, as befits his or her professional responsibility, ultimately arrives at an estimate of risk to human health.

Establishing a Comparison

Some criteria of the validity of animal data for use in human risk assessment are presented in Table 1. For any experiment, the report itself provides internal evidence of the quality of work performed and of the individuals involved. With the advent of regulations defining good laboratory practice, considerable improvements in the conduct of laboratory studies may be anticipated. Nevertheless, such is the variety and complexity of toxicological investigations that prescribed procedures can cover only those aspects that are necessary for regulatory agency and legal purposes.

Before attempting to establish a comparison, it is advisable to ascertain the meaningfulness of the test

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Table 1. Validity of animal data.

Quality of laboratory:	Personnel, facilities, animals, animal care, records
Protocol:	Design and execution (sample size, dose, duration)
Pathologists:	Input, gross and microscopic appraisals
Quality of data:	Internal evidence Dose-response characteristics Consistency, reproducibility Loose ends

or tests under consideration. One example is the production of terata in chick embryos by injection of foreign materials into the hen's egg. Striking effects may be elicited, even by grains of sand. It is questionable whether relevant conclusions concerning mammalian reproductive effects can be drawn from a closed system like the avian egg (1). Another test that requires cautious interpretation involves repeated subcutaneous or intramuscular injection of the test compound in high doses into rats or mice (2). Iron dextran complex, introduced into clinical medicine in 1954, was later shown to produce a variety of sarcomas in high yields under these conditions (3-5); 25 years and millions of patients later, not a single human case of sarcoma or other form of neoplasia has been shown to be clearly attributable to iron dextran (6).

Such considerations are appropriate at this time, in view of the widespread eagerness — amounting in some instances to ill-advised haste — to adopt newly-developed “short-term” tests (of mutagenic potential) for regulatory purposes. Until such tests are adequately validated, their suitability, reliability, reproducibility, precision, sensitivity, and specificity are open to question (7). The current international effort at validation, after more than two years of intensive work, has served to demonstrate the difficulties that still exist in standardizing the procedures used in even the most (superficially) “simple” short-term tests (8).

The assessment of severity of a toxic effect often goes beyond readily-quantifiable measurements (Table 2). The general characteristics of the changes elicited should be taken into consideration; beyond those, the nature of the target tissue is a critical factor. In the absence of human data to indicate with some certainty the likely human target organ, one has at least to question whether the animal findings are likely to be applicable to man. A striking example of a structure that does not exist in man is the tapetum lucidum or nictitating membrane in the eyes of dogs and cats. The ocular toxicity of hydroxy-pyridinethione and zinc pyridinethione, which produce blindness in dogs, is attributable to an action on

the tapetum. Atapetal dogs and rhesus monkeys suffer no ill effect (9). The drug ethambutol, among other zinc-chelating compounds, is a similar case in point (10-13). In neither case is there any evidence of an ocular effect in man.

The manifestations of systemic toxicity that need to be taken into account in assessing severity are indicated in Table 2. The nature and extent of damage brought about, and the particular tissue in which it occurs, are key considerations. A discussion along these lines has been published (14), in which the issues of regeneration, repair and restoration of function are categorized for various tissues: those having little or no capacity for regeneration (repair through fibrous replacement, as in nerve and muscle cells, including myocardium); those whose cells do not normally replicate but retain the ability to do so in response to damage (liver, kidney, type 2 alveolar epithelium); and labile tissues that continually proliferate and replace themselves (surface epithelia and hemopoietic tissue). Cumulative, progressive and irreversible effects should be weighed, particularly in the first of these tissue categories. The issue of irreversibility, however, calls for caution, since there is increasing evidence of the apparent reversibility (on discontinuation of exposure) of early changes brought about by carcinogens, for example, in bladder epithelium and liver (15, 16).

A Judgment is Rendered

The relevance of animal data for human risk assessment is based on a variety of considerations set out in Table 3. Some aspects have been discussed above. Of the remainder, metabolism and pharmacokinetics are of major importance. A detailed discussion of this area of toxicology has been published recently (17). In attempting to render a judgment on human risk, the question of “saturation

Table 2. Criteria of severity of toxic effect.

General characteristics	
Acute:	Reversible or irreversible
Chronic:	Progressive, cumulative, reversible or irreversible
Mutagenesis, carcinogenesis	
Reproduction, development, teratogenesis	
Immunologic effects	
Behavioral changes	
Nature of target tissue	
Reserves of function	
Capacity for regeneration, repair, restoration of function	
Nature and extent of damage	
Changes in organ function (and other tests)	
Liver, kidney, bone-marrow	
Special function tests	

Table 3. Relevance of animal data for human risk assessment.

Limitations of animal data
Species and strain differences
Genetic uniformity
Diet, controlled environment
Gnotobiotic state
Specificity of toxic action(s)
Interspecies differences
Relevance of affected organ
Metabolism and pharmacokinetics
Interspecies comparisons (including humans)
Dose relationships to metabolic characteristics and impact on defense mechanisms
Parallelism of covalent binding and toxicity.

point" is vital. This refers to the level of exposure in a saturable, dose-dependent metabolic process at which the capacity of the body's defensive mechanisms is exceeded. In consequence, the linear, first-order kinetics change to zero-order kinetics (18-21). Unfortunately, little is known quantitatively about the human body's response with regard to capacity to maintain defensive levels of glutathione and other nonprotein sulfhydryl compounds in various organs in the face of mounting exposure to toxicants, or their chemically reactive metabolites, that require detoxification by a route involving thiol derivatives. Equally, the methods developed for detection and measurement of covalent binding of chemically reactive metabolites to cellular protein, phospholipid, DNA, and RNA are not readily applicable to noninvasive human studies. Osterman-Golkar and her colleagues (22) and Truong, Ward, and Legator (23) have made efforts in this direction, but much remains to be done. That a high priority should be allotted to such work is evidenced by observations on the parallelism between the amount of vinyl chloride metabolized or the extent of covalent binding of labeled vinyl chloride metabolites to liver protein, and the incidence of angiosarcoma of the liver in rats (19).

Analysis of the implications for human health (Table 4) should take into account differences in human susceptibility (Table 5), which are based partly on ability to adapt to exposure, metabolic capacity, detoxication potential and reserves of function. Assessment of the extent of exposure of the population is often difficult, and calls for arbitrary decisions. Where exposure is partly or entirely through food, the so-called "glutton syndrome" enters into the picture and allowance should be made for the 90th percentile of consumption.

The need for information on human exposure to a compound and its effects is self-evident; but the quest for such data is often difficult and calls for determination and dogged persistence. Of the cate-

Table 4. Analysis of implications for human health.

1. Results of toxicity and other studies in animals
2. Quantitative data on actual or potential human exposure
3. Data on human health effects
4. Statistical extrapolations from dose-response relationships to levels of human exposure

gories of human information that may be available (17, 24), the results of human exposure under controlled conditions are particularly valuable. For the most part, however, epidemiological studies are the source of human information usually relied upon. Unfortunately, the bias of the cancer enthusiasts is such that positive results of epidemiological studies are emphasized but negative findings are subjected to nitpicking which, ipso facto, renders them "controversial" and hence capable of being safely disregarded. Conversely, case reports or epidemiological associations, however tenuous, suffice to label a compound as a human carcinogen, irrespective of the number and high quality of negative long-term studies in animals (25).

An instructive example of positive findings in animal carcinogenesis bioassays is that of phenobarbital, which has yielded hepatic adenomas and carcinomas in mice and limited evidence of benign liver tumors in rats (26-30). By today's standards, phenobarbital would not be accepted for use as a drug. Happily, half a century of experience of its value and safety in the treatment of epilepsy has afforded an opportunity for a well-conducted epidemiological study carried out by Clemmesen and Hjalgrim-Jensen (31), who followed up a total of 9136 epileptic patients admitted to the epileptic colony at Filadelfia, in Denmark, over the period 1933-1962. Up to December 1972, after making allowance for patients in whom thorotrast had been used for angiography, three cases of liver cancer were found, as against 2.1 cases expected. The excess of brain tumors was just that to be anticipated in epileptic individuals. It is worth quoting Clemmesen's *cri de coeur* (31)

"... if the evidence presented here for 8,078 persons followed for one or two decades should carry no weight in the discussion on the postulated carcinogenicity of phenobarbital we may as well abandon cancer epidemiology."

Is phenobarbital acting as a modifying factor for

Table 5. Differences in host susceptibility.

Genetic factors
Constitutional factors
Dietary factors
Life style: alcohol, tobacco, exercise, drugs
Occupational exposures
Environmental exposures

endogenous carcinogenic influences, for example, cancer viruses, that are present in mice? This possibility, linked to the fact that Clemmesen's studies were carried out in human adults, and that brain tumors in children may differ etiologically from those in adults, led Gold and her colleagues (32) to study the risk of brain tumors in children exposed to barbiturates. Their results suggested that up to 8% of brain tumors in children may be attributable to ingestion of barbiturates by the children themselves or, prenatally, by their mothers.

An important step in hazard analysis is the comparison of the risk under consideration with the general background of risk to man from "natural" foods, air and water, just as low-level radiation hazards are judged against the natural background of cosmic and other radiation. Strangely enough, no attempt has ever been made to assess background chemical risk from the natural environment, to which man has presumably been exposed from time immemorial. Yet, estimates of this kind are vitally necessary in risk assessment, even for products of combustion entering the atmosphere. From the food additive area comes a current illustration. Nitrite used as an additive in the preparation of cured meats seems likely to be banned in the near future. Yet, the nitrite in cured meat constitutes at most 2-3% of the total nitrite to which the body is exposed; 82% is formed directly in the human intestine (33), and 15% enters through the saliva as a result of conversion of dietary nitrate to nitrite by microorganisms in the buccal cavity. What benefit to anyone is likely to flow from regulatory action to eliminate, on average, 1% of nitrite exposure? On the basis of measurements of volatile nitrosamines in human blood and feces, the calculated daily human exposure to dimethylnitrosamine is in the range 500-5000 μg ; meat products contribute 0.5 μg and 30 cigarettes 0.6 μg (34). As far as we can tell, most of the rest of these carcinogens are synthesized within the body (35).

What lessons flow from these examples? Hazard analysis must consider three components: potential, the intrinsic ability of a compound to elicit toxic effects, for instance, mutagenic/carcinogenic changes; potency, which covers a range of at least 10^7 ; and opportunity, i.e., exposure, with a range of at least 10^8 . Since the two latter factors are independent of each other, the total potency associated with exposure covers a dose range of 10^{15} . What this means is that there is a need to discriminate between compounds at the upper end of this enormous spectrum and those at the lower end. In taking appropriate precautions, the approach that would treat all alike suffers from the disadvantage that many compounds will be subject to excessive or even super-

fluous restrictions whereas the really bad actors may not be covered strictly enough.

In real life, the range of potency \times exposure is likely to exceed 10^{15} because of mitigating factors (physiological and homeostatic mechanisms, interactions, competition, detoxication) modulating factors (effects on metabolism, DNA repair, immune response) and modifying factors influencing mutagenic, carcinogenic and other responses. Thus, the total impact of the compound may be expressed in terms of an unsolved equation (36), in which the first term contains an unknown coefficient multiplied by the biological activity of the compound, for instance its carcinogenic potential. To this term must be added others, representing the products of unknown coefficients and the activities of (a) syncarcinogens, cocarcinogens, promoters and modifying factors, (b) carcinogens present in "natural" foods and (c) carcinogens formed endogenously by the human body. Terms to be subtracted are the products of unknown coefficients and (a) the protective actions of bodily defense and adaptive mechanisms, (b) the activities of anticarcinogens and other tumor suppressants present in and entering the human body. While such a solution is probably a pipedream at present, efforts in this direction will make for better and better hazard evaluations.

Conclusion

To analyze the implications for human health, the toxicologist requires four sets of data: the results of toxicity and other studies in animals; quantitative data on actual or potential human exposure; whatever information is available on effects of exposure in man; and the statistical extrapolations from the dose-response relationships in animals to the (usually) much lower levels of human exposure. Professional expertise in toxicology is essential to assess the nature and severity of the toxic effects observed in animals, including such characteristics as potential for progression, irreversibility and production of incapacity. Given sufficient data, an estimate can be arrived at of the likelihood that such effects will be elicited in human populations of differing susceptibilities. The criteria by which the overall implications for human health can be judged comprise both the direct effects on man, as well as the indirect consequences stemming from environmental impacts.

This presentation has deliberately avoided such controversial issues as that of thresholds for mutagens/carcinogens, or the weaknesses of carcinogenesis bioassays in laboratory animals. Instead, emphasis has been placed on commonsense

issues on which general agreement may be anticipated. One of these is the dictum that scientific judgment should rest on the weight of scientific evidence; compound character assassination is not good toxicology. Equally, the implications of animal data for human health can only be assessed by scientists — not lawyers — on a solid scientific basis. A physician can advise, but is not trained to interpret toxicological data. A lawyer can do neither. In this context, there is an important difference between science and law in regard to the evidence needed for attribution of causality to statistical association between postulated causes and observed effects.

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